Announcement of the BioSync web site

We are pleased to announce the BioSync Web resource at *http://biosync.sdsc.edu*. Developed on behalf of the Structural Biology Synchrotron Users Organization (BioSync), this resource serves as a structural biologist's guide to synchrotron facilities. The BioSync organization has collaborated with the San Diego Supercomputer Center to create a comprehensive synchrotron portal and informational web site using modern information practices. Users of the web site can investigate the different technical capabilities of each beamline and find out logistical information such as scheduling and travel arrangements. Synchrotron personnel update the web site regularly to provide current information to the user community and to help guide projects to the appropriate facility. In addition, BioSync is working with the Protein Data Bank (PDB; *http://www.rcsb.org/pdb*) to automatically include beamline specific information with structure depositions. The BioSync web resource strives to provide timely, accurate, and complete information on synchrotron resources to the community of structural biologists. This resource is a part of the National Biomedical Computation Resource, an NIH National Center for Research Resources hosted at the San Diego Supercomputer Center.

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Questions about the structure of the botulinum neurotoxin B light chain in complex with a target peptide

Hanson and Stevens[1] recently reported the cocrystal structure of the botulinum neurotoxin B light chain in complex with a 38-residue target peptide of synaptobrevin II. Based on a careful examination of the published data, we have serious concerns about the strength of experimental evidence supporting the presence of the target peptide, and consequently the validity of inferences about substrate binding made in the report.

The experimental B-factors (PDB entry 1f83) are excessively high for the substrate atoms. Their average B-factor is 128 Å^2, approximately four times the average B-factor for the protease atoms. While B-factors alone do not provide a definitive measure of quality for model features, excessive B-factors for the peptide substrate in this case raise warning signs.

In addition to having excessive B-factors the substrate model does not conform to expected stereochemical restraints. Conformational analysis of the peptide reveals only a few of the 36 modeled peptide residues in most favored regions of the Ramachandran plot while seven peptide residues fall in disallowed regions. While the enzyme may induce a strained peptide conformation, there are scarcely few strong contacts between substrate and enzyme to stabilize such a high-energy conformation. Furthermore, there are few

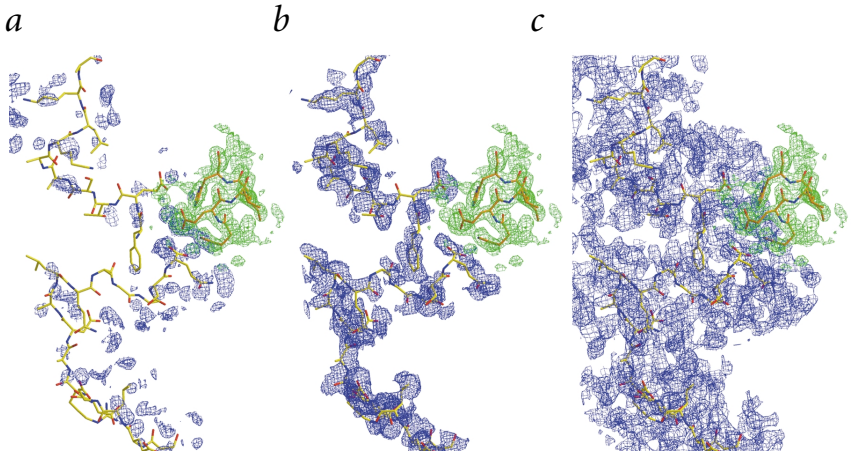

Fig. 1 Electron density for modeled substrate synaptobrevin II and BotB light chain. Orientation of the model fragments, map contour levels and colors approximate Fig. 2 of Hanson and Stevens[1]. ***a***, Electron density of a wARP[3] map. Displayed in blue (1.2 σ level) and within 4 Å (using the 'blob' contouring feature in XtalView[6]) is electron density of the modeled substrate. Shown in green, electron density of the zinc binding HEXXH motif of the botulinum B light chain, but contoured at 2.0 σ and within 3 Å of the binding motif. The wARP[3] map reveals strong and clear electron density that agrees with the enzyme model (green contours) whereas very little electron density for the substrate (blue contours) appears even at the lower contour level. No connectivity from which the features of the modeled substrate could be confirmed is evident. ***b***, CNS[5] σ_A[4] weighted $F_o - F_c$ omit electron density map superimposed on the modeled substrate, with display parameters chosen to generate a view of electron density apparently confirming the presence of the modeled substrate. This electron density map was made by selecting a high grid density for smoothness, a relatively low contour level for connectivity (matching the reported[1] contour level of 1.2 σ), and with a small cutoff radius (1.6 Å) using the 'blob' feature in XtalView[6] to force conformity of the map with the model. ***c***, Same CNS σ_A weighted $F_o - F_c$ omit map as in (*b*), same contour level as reported[1], but with a larger volume displayed. The 'blob' cutoff radius is now set to 4 Å and the electron density map reveals many features not related to the modeled substrate and poor connectivity. At this contour level, the map shows many large positive features even in the core of the protein, indicating that the map is in fact contoured at noise level. From inspection of panels (*a*) and (*c*) we do not believe that it is possible to discern details of the interaction between substrate and enzyme such as are shown in Fig. 3 of Hanson and Stevens[1].

significant changes to the modeled enzyme upon substrate binding (root mean square (r.m.s.) deviation between free and substrate bound enzyme is 1.7 Å for all atoms and 0.3 Å for all Cα atoms; r.m.s. deviation is 0.2 Å for all atoms and 0.09 Å for Cα atoms for residues near the catalytic site). Even considering that a low or partial occupancy (likely due to the short soaking times for the large peptide) could give rise to weak data and high B-factors, the peptide should exhibit plausible stereochemistry.

Most significantly, deposited structure factors (r1f83sf.ent) and model coordinate (1f83) provide a means for inspecting the primary experimental evidence (electron density maps) for the modeled substrate. We have constructed (in order from least to most model biased) Shake&wARP (Fig. 1*a*), σ_A weighted $2F_o - F_c$ omit, σ_A $F_o - F_c$ omit, $F_o - F_c$ omit, and $2F_o - F_c$ maps from the deposited data in an effort to generate electron density as shown in Fig. 2*a* of the paper by Hanson and Stevens[1]. Despite our substantial efforts to confirm the published results we have found no electron density above noise supporting any definite conclusions about the substrate (independent analysis confirming weak real space correlation and Z-scores is available from the Uppsala electron density server[2]). Regardless of the procedure or programs used (wARP[3], σ_A[4], CNS[5], XtalView[6]/SHELXL[7]), none of the maps that we have produced reveals electron density of the apparent clarity shown in the published report at the reported contour levels (Fig. 1*c*). While weak difference density appears in some areas, possibly due to a low occupancy ligand, we do not believe that any detailed inferences about the nature, conformation, or binding mode of the ligand can be made with any certainty.

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Response to Rupp and Segelke

In response to the Comment by Rupp and Segelke concerning our paper published in *Nature Structural Biology* last year, we apologize for omitting that the occupancy of the peptide is estimated to be ~30–40%. Our published study was a rapid soak freeze-trap experiment, and in order for us to observe the product bound form of the peptide, it is not unexpected that the peptide is of lower occupancy in the crystal than the protein molecule. However, we should have noted this in our publication. The stereochemical quality of the peptide is low, and we believe this to be due to the occupancy of the peptide. However, since the peptide is disordered in solution, we did not want to constrain the peptide to an artificially high quality stereochemical model. Attempts to obtain higher occupancy binding of the peptide were not successful. The peptide is most clearly observed when one compares the active site of both the apo (1f82.pdb, r1f82sf.ent) and peptide bound (1f83.pdb, r1f83sf.ent) forms. This latter comparison helps to resolve any potential phase bias that might have occurred from the molecular replacement solution using the holotoxin model.

More germane to the science is the discovery that the synaptobrevin peptide binds in the same location as the translocation domain of the holo-botulinum neurotoxin after translocation domain separation. This observation is consistent with published biochemical studies as cited in our publication and it provides a potential explanation for previously unexplained observations in the field of botulinum neurotoxin research. Additional biochemical data are accumulating that support our observation and I suspect we will read more articles in the near future on this topic. Based on our 2.2 Å apo and 2.0 Å substrate-bound structures and additional structural studies that we have conducted since publication, we continue to support our published proposal on how the neurotoxin cleaves synaptic vesicle proteins. However, our goal is to accurately understand how the neurotoxin works and if our proposal is not correct, we would like to know as soon as possible to advance the field in a forward direction. We look forward to seeing this matter resolved in the peer-reviewed literature in the near future.

In summary, we thank Rupp and Segelke for pointing out that we did not state the occupancy of the peptide in our published article, and we apologize to the community for this omission.

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